OIPE
JUL 25 2005
JC50
PATENT & TRADEMARK OFFICE

AF/1644

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Application Number	:	09/381,032	Confirmation No. 3417
Applicant	:	Andreas Bergmann et al.	
Filed	:	December 17, 1999	
Tech Cntr/AU	:	1644	
Examiner	:	Phuong N. Huynh	
Entitled	:	Receptor Binding Assays for the Detection of TSH Receptor Autoantibodies and Reagents and Reagent Kit for Carrying Out Such a Receptor Binding Assay	
Attorney Reference	:	121778-40263260	
Customer Number	:	43569	

MAIL STOP APPEAL BRIEF - PATENTS
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

TRANSMITTAL FOR APPEAL BRIEF

Transmitted herewith is an appeal brief for this application.

SMALL ENTITY STATUS

Applicant confirms that small business entity status is claimed in this application.

EXTENSION OF TIME

A petition for extension of time under 37 C.F.R. 1.136 for one month (PTO/SB/22) is attached hereto in duplicate.

ADDITIONAL FEES

Applicants also submit herewith the following fees:

Filing fee for a Brief in Support of an Appeal (Fee Code 2402)	$250.00

FEE PAYMENT

Authorization is given to charge the fees itemized herein and the attached extension of time fee, as well as to charge any deficiencies in the fees, and/or to further credit any overpayments, to Deposit Account No. 503-121 to maintain the pendency of this application. A duplicate copy of this transmittal is attached hereto.

Respectfully Submitted,

MAYER BROWN ROWE & MAW LLP

By: Richard A. Steinberg

Richard A. Steinberg
Registration No. 26,588

Intellectual Property Group
1909 K Street, N.W.
Washington, D.C. 20006
(202) 263-3000 Telephone
(202) 263-3300 Facsimile

Date: July 25, 2005

OIPE JC50 JUL 25 2005 PATENT & TRADEMARK OFFICE

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Application Number	:	09/381,032	Confirmation No. 3417
Applicant	:	Andreas Bergmann et al.	
Filed	:	December 17, 1999	
Tech Cntr/AU	:	1644	
Examiner	:	Phuong N. Huynh	
Entitled	:	Receptor Binding Assays for the Detection of TSH Receptor Autoantibodies and Reagents and Reagent Kit for Carrying Out Such a Receptor Binding Assay	
Attorney Reference	:	121778-40263260	
Customer Number	:	43569	

MAIL STOP APPEAL BRIEF - PATENTS
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPELLANT'S BRIEF UNDER 37 C.F.R. § 41.37

Sir:

Further to the Notice of Appeal under 37 C.F.R. 43.31 filed April 25, 2005, Bergmann *et al.*, hereinafter "Appellant," appeals the decision, finally rejecting claims 23-25 and 27-33, dated January 24, 2005 of the Primary Examiner. This appeal is taken with respect to the finally rejected claims 23-25 and 27-33, as amended in the Amendment and Response filed October 28, 2004.

The requisite fee required under 37 C.F.R. 41.20(b)(2), as well as any additional fees necessary to continue the prosecution of this application, is authorized on the attached transmittal sheet to be charged to Deposit Account 503-121.

07/27/2005 MAHMED1 00000075 503121 09381032
01 FC:2402 250.00 DA

(i) ***Real party in interest***

The assignee of the subject application and the real party in interest is B.R.A.H.M.S Aktiengesellschaft.

(ii) ***Related appeals and interferences***

There are no related appeals or interferences.

(iii) ***Status of claims***

Claims 23-25 and 27-33 were finally rejected. The application was originally filed with claims 1-13. Claim 13 was withdrawn from prosecution following a requirement for restriction. Claims 14-22 were added and claims 1-12 were cancelled in an amendment filed November 2, 2001. Claims 23-32 were presented and claims 14-22 were cancelled in an amendment filed October 23, 2002. Claims 23 and 24 were amended, claim 26 was cancelled and claim 33 was added in an amendment filed on February 2, 2004; and subsequent claim amendments were presented in the amendment filed on October 28, 2004.

This appeal is taken with respect to the finally rejected claims 23-25 and 27-33, as amended on October 28, 2004, prior to the date of the Final Rejection, as further elucidated in the Advisory Action Before the Filing of an Appeal Brief, dated June 15, 2005.

Claim 13, although not referenced in any Office Action letter, subsequent to the Office Action dated September 2, 2003, has not been formally cancelled and stands as withdrawn.

(iv) ***Status of amendments***

No claim amendment was filed subsequent to the date of the Final Rejection.

(v) ***Summary of claimed subject matter***

The claimed subject matter, as set forth in independent claim 23, relates to a method for the determination of thyroid stimulating hormone (TSH) receptor autoantibodies. The autoantibodies against the TSH receptor play a role in a number of thyroid disorders, such as, for example, Graves" disease (page 1, lines 6-9). The inventive receptor binding assay is a solid phase assay. The claimed receptor binding assay is one in which the TSH receptor

complexes formed from the reactants of the assay are obtained directly in a form bound on the solid phase, whereby an automated procedure becomes feasible (page 11, lines 28-34).

According to a first step (i) of the claimed method, a solid phase (*see, e.g.*, page 20, lines 12-16), comprising an affinity-purified immobilized functional recombinant human TSH receptor [rhTSHR(imm)*] (page 16, lines 14-19; page 18, lines 16-20; page 32, lines 1-28), is reacted with a liquid biological sample to be assayed for the presence of the TSH receptor autoantibodies (page 11, lines 23-34; page 18, lines 16-24).

According to a second step (ii) of the claimed method, the reacted solid phase is separated from the liquid biological sample (page 20, lines 9-11 and 22-30; page 33, line 23 to page 34, line 20).

According to a third step (iii) of the claimed method, the reacted solid phase is washed (page 34, line 4, lines 12-15).

According to a fourth step (iv) of the claimed method, the reacted solid phase is incubated (page 34, lines10-11) in the presence of a buffer solution (page 34, lines 5-9). The buffer solution comprises an amount of labeled bovine TSH (bTSH) (page 13, lines 24-33 to page 14, lines 1-7; page 23, line 27 to page 24, line 14; page 34, lines 5-9). Incubation is allowed to proceed for a sufficient time to occupy all of the TSH binding sites of the functional rhTSHR not occupied by the autoantibodies (page 34, lines 10-11).

According to a fifth step (v) of the claimed method, the presence and/or amount of the autoantibodies is determined. The determination is made on the basis of the amount of labeled bTSH bound to the solid phase (page 34, lines 16-20).

The claimed method is further characterized by the use of a selective monoclonal antibody for immobilization of the functional rhTSHR (page 32, lines 1-28). The selective monoclonal antibody is one that recognizes only conformational epitopes of the human TSH receptor (page 30, lines 31-35; page 31, lines 1-21). The monoclonal antibody is obtained by immunizing an animal with a DNA plasmid construct encoding the human TSH receptor (page 10, line 28 to page 11, line 4; page 30, line 31 to page 31, line 5) and washed (page 16, lines 14-19; page 32, lines 20-26).

According to the embodiment of the invention as set forth in independent claim 24, the determination of TSH receptor autoantibodies (TSH-auto-ab) is accomplished by a method according to which, in a first step, (i) a solid phase (page 20, lines 12-16), comprising

an affinity-purified immobilized functional recombinant human TSH receptor [rhTSHR(imm)*] (page 16, lines 14-19; page 18, lines 16-20; page 32, lines 1-28; page 33, lines 2-9), is reacted with a solution prepared from:

(a) a serum-containing biological sample to be assayed for the presence of the autoantibodies (page 11, lines 23-34; page 18, lines 16-24; page 33, lines 7-9), and

(b) a buffer solution containing an amount of labeled bovine TSH (b-TSH) for a sufficient time to occupy all the TSH binding sites of the functional recombinant human TSH receptor not occupied by the autoantibodies (page 32, line 29 to page 34, line 20; page 33, lines 10-14).

According to a second step (ii) of this embodiment, the solution is removed from the reacted solid phase (page 33, lines 15-16).

According to a third step (iii) of this embodiment, the reacted solid phase is washed (page 33, line 17).

According to a fourth step (iv) of this embodiment, the presence and/or amount of the autoantibodies are determined on the basis of the amount of labeled b-TSH to the solid phase (page 33, lines 18-22).

The method according to this embodiment of the invention is further characterized by immobilization of the functional rhTSHR(imm*) (page 16, lines 14-19; page 18, lines 16-20; page 32, lines 1-28), to the solid support by a selective monoclonal antibody that recognizes only conformational epitopes of the human TSH receptor (page 17, lines 13-16; page 32, lines 1-28; page 30, lines 31-35; page 31, lines 1-21). The monoclonal antibody is obtained by immunizing an animal with a DNA plasmid construct encoding the human TSH receptor (page 25, lines 7-10; page 30, line 31 to page 31, line 21) and washed (page 16, lines 14-19; page 32, lines 20-26).

In another embodiment of the invention, as represented by independent claim 33, a method for determination of TSH receptor autoantibodies includes at least the following steps:

(i) reacting a solid phase (page 20, lines 12-16), comprising an affinity-purified immobilized functional recombinant human TSH receptor [rhTSHR(imm*)] (page 16, lines 14-19; page 18, lines 16-20; page 32, lines 1-28), with a liquid

biological sample to be assayed for the presence of the autoantibodies (page 11, lines 23-34; page 18, lines 16-24);

(ii) separating the reacted solid phase from the liquid biological sample (page 20, lines 9-11 and 22-30; page 33, line 23 to page 34, line 20);

(iii) washing the reacted solid phase (page 34, line 4, lines 12-15);

(iv) incubating the reacted solid phase with a buffer solution comprising an amount of labeled bovine TSH for a sufficient time to occupy all the TSH binding sites of the functional recombinant human TSH receptor not occupied by the autoantibodies (page 34, lines 5-11); and

(v) determining the presence and/or amount of the autoantibodies on the basis of the amount of labeled bovine TSH bound to the solid phase (page 34, line 21 to page 38, line 3);

wherein the functional recombinant human TSH receptor is immobilized to a solid support by a selective monoclonal antibody that:

a) recognizes only conformational epitopes of the functional human TSH receptor and is obtained by immunizing an animal with a DNA plasmid construct encoding the human TSH receptor (page 10, line 28 to page 11, line 4; page 16, lines 14-19; page 18, lines 16-20; page 30, lines 31-35; page 31, lines 1-5; page 32, lines 1-28; page 17, lines 13-16; page 32, lines 1-28); and

b) does not bind to peptides representing short sequences of the human TSH receptor, but which shows strong binding to the complete functional recombinant human TSH receptor (page 30, line 31 to page 31, line 5; page 31, lines 15-21);

and then washed (page 16, lines 14-19; page 32, lines 20-26).

(vi) ***Ground of rejection to be reviewed on appeal***

The ground of rejection to be reviewed is whether the Examiner has erred in maintaining the final rejection of claims 23-25 and 27-33, under 35 U.S.C. 103(a), as being unpatentable over Vitti *et al.* (Acta Med Austriaca 23 (1-2): 52-56, 1996) in view of Harlow *et al.* (Antibodies A Laboratory Manual, Cold Spring Harbor Laboratory, pages 556, 564-

591, 1988) and Nicholson *et al.* (J. Mol. Endocrinol. 16(2): 159-170, 1996) or Morgenthaler *et al.* (J. Clin. Endocrinol Metab. 81(2): 700-706, Feb. 1996).

In this regard, Appellants understand that the rejection in part 6, beginning on page 9 of the Final Rejection (January 24, 2005) has been withdrawn insofar as this rejection, which relies on U.S. Patent 5,614,363, as the primary reference, is not addressed in the Advisory Action Before the Filing of an Appeal Brief (June 15, 2005). It is presumed that the Examiner was persuaded by at least the arguments presented in the Amendment and Response filed October 28, 2004, at page 7, which fully addressed this rejection.

If, however, Appellants have misunderstood the Examiner's intentions, than a further issue to be reviewed on appeal is whether the Examiner has erred in rejecting claims 23-25 and 27-33, under 35 U.S.C. 103(a), as unpatentably obvious over U.S. Patent 5,614,363, in view of Vitti *et al.* (Acta Med Austriaca 23 (1-2) 1996: 52-56), Harlow *et al.* (Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, pages 556, 564-591, 1988) and Nicholson *et al.* (J. Mol. Endocrinol. 16(2) 1996: 159-170) or Morgenthaler *et al.* (J. Clin. Endocrinol Metab. 81(2): 700-706, Feb. 1996).

(vii) ***Arguments***

Rejection of claims 23-25 and 27-33 under 35 U.S.C. 103(a) as unpatentable over Vitti in view of Harlow and Nicholson or Morgenthaler

The present invention, as defined by the claims presented on appeal, is directed to a method for the determination of pathological autoantibodies against the TSH receptor which play a role in a number of thyroid disorders, the most prominent of which includes Graves' disease.

Prior to the present invention, the dominant assay for clinical settings (the TRAK assay), as described in the introductory part of the specification, was an assay which relied on the competitive binding of the population of the autoantibodies being measured and labeled bovine TSH (bTSH) to a solubilized porcine TSH receptor, followed by a final precipitation step. TRAK assays, also known as TBII assays, are based on the competition between the autoantibodies to be determined with labeled TSH (labeled bTSH) for the same binding sites (epitopes) or binding sites in close (spatial) proximity, and, therefore, *per se*, can only detect autoantibodies which are, in fact, competing with TSH.

The prior art TRAK assay did not "find" the autoantibodies to be detected in about 20% of the patients who in fact had such autoantibodies (*e.g.*, had Graves disease – GD). That is, while all patients determined to be ill by the TRAK assay were, in fact ill, *e.g.*, had GD, (*i.e.*, 100% specificity), the "selectivity" was only about 80% (only about 80% of the GD patients were identified).

The present inventors sought to overcome this "selectivity" problem of the TRAK assay. Whereas the original TRAK assay used a solubilized TSH receptor (meaning that essentially the complete surface of the receptor floats in the liquid reaction medium is freely available and accessible to all reagents from all sides and not occupied by interfering reagents.

In contrast, the present methodology provides a "solid-phase" assay which avoids the necessity for the precipitation step. At the end of the assay, the resulting proportional amount of bound label bound to a solid phase, *e.g.*, a coated tube, can, after separation of the solid from the liquid reaction mixture, be directly introduced into the measurement procedure or device.

In order to achieve a successful solid phase assay it was necessary to immobilize the TSH receptor. To do this, as will be readily appreciated, something must come into contact with (*i.e.*, become attached to) a surface of the receptor to affix it to the solid phase. Those portions of the surface which are contacted by the immobilizing agent, and certain adjacent portions of the TSH receptor facing the solid phase become inaccessible for the reagents, *i.e.*, they are lost for the analytical binding reaction. This effect, together with (assumed) undesirable structural changes of the TSH receptor, due to the delicate nature of this molecule, led to, e.g., loss of functionality, resulting in the loss of specificity of the binding of the target autoantibodies - as well as to bovine TSH. Thus, prior to the present invention, attempts to create a solid phase assay by immobilizing a TSH receptor, resulted in loss of sensitivity. Therefore, the difficulty in immobilizing the TSH receptor precluded development of a solid phase assay which could compete with, or that was of better quality than, the existing precipitation assays.

The present invention overcomes the problem of loss of functionality and provides the first effective solid phase assay methodology for the determination of TSH receptor autoantibodies.

The method, as set forth in the claims presented in this appeal, may be characterized as a "second-generation" for TSH receptor autoantibodies (TSHR-auto-ab) and provides unexpectedly superior results to the "first-generation" or precipitation-type assays.

In the various embodiments of the method according to the present invention which are involved in this appeal, a functional recombinant human TSH receptor is immobilized by a special type of monoclonal antibody, namely, one which recognizes only conformational epitopes of the TSHR and, is obtained by immunization of an animal with a TSHR DNA construct; they are not obtained by traditional immunization techniques, for example, those using complete hTSHR or portions of hTSHR as antigen.

As will become more readily apparent from a comparison of Appellant's invention, as represented by the embodiments encompassed by the claims involved in this appeal, with the known prior art techniques for assays for the determination of TSH receptor autoantibodies, the Appellant's invention has at least the following unique characteristics:

(i) a functional recombinant human TSH receptor (rhTSHR) is used as selective binder for both the autoantibodies and for the labeled marker substance (labeled bovine TSH);

(ii) the rhTSHR is provided in immobilized form (rather than in solubilized form);

(iii) the sensitivity of the rhTSHR to structural changes resulting in loss of specificity of binding as a result of its immobilization to the solid support is overcome by using a special type of antibody to effect the immobilization. In particular, the special antibody is a monoclonal antibody which recognizes only conformational epitopes of the human TSHR. These monoclonal antibodies are not obtained by traditional immunization techniques using complete or portions of hTSHR as "antigen" for immunization. Rather, as set forth in the appealed claims, a "DNA construct" which contains the complete cDNA of the hTSHR, is used for immunizing an animal, *e.g.*, mouse, host.

In this regard, whether or not such unique monoclonal antibody is, *per se*, novel, is not the issue for this appeal. Rather, as will be apparent from review of the prior art, there is no disclosure in any prior art which even remotely suggests that the existence of monoclonal antibodies which would have the ability to immobilize a functional rhTSHR without causing

the usual loss of functionality, nor is there any suggestion or motivation to use such monoclonal antibodies for immobilizing the rhTSHR. In particular, nothing in the prior art suggests that the sensitivity of a receptor binder, such as TSHR, to structural changes resulting from attempts to immobilize the receptor to a solid phase can be solved by using a special monoclonal antibody to effect the immobilization.

In this regard, antibodies which are useful for immobilization needed to be found based not only on conformational nature, as such, since there may be conformational antibodies obtained by conventional immunization techniques which in fact block TSH binding or autoantibody binding and, therefore, would be useless for the development of a "solid phase TRAK assay." Indeed, mAb A9, of the cited prior art, may be such an antibody. Rather, a successful antibody would be one in which its peculiar way of binding to the TSH receptor did not also result in blocking or otherwise affecting those surface regions of the human TSH receptor important for the competition of labeled TSH with the great variety of autoantibodies found in sera of human GD patients.

Absent knowledge of the existence of such unique conformational monoclonal antibody the practitioner would not have been motivated to look for such a molecule nor would the practitioner have had a reasonable expectation of success in finding an agent for immobilization of a functional rhTSHR in a solid phase assay for TSH receptor autoantibodies. Certainly, the prior art does not offer any disclosure or suggestion that a conformational monoclonal antibody raised by immunization of an animal with a DNA plasmid construct encoding the human TSH receptor, would behave differently, much less, with superior effect, to sequential or other types of antibodies for immobilizing functional rhTSHR. This deficiency in the prior art should, without more, require reversal of the final rejection of claims 23-25 and 27-33.

As also evident from embodiments of the invention which are involved in this appeal, *e.g.*, see claim 23, Appellant's method involves a "two-stage" assay (see, also, for example, the specification beginning at page 33, line 23). According to this embodiment, the reaction of the immobilized functional rhTSHR with the autoantibodies in a sample of biological liquid and the subsequent reaction with labeled (bovine) TSH, are separated by a washing step. As explained at page 34, line 24 to page 35, line 7, in the two-stage assay, the labeled bTSH preparation may be serum-free (*e.g.*, claim 28). In addition, however, the use of

labeled bTSH in a second assay step, in the absence of unbound sample constituents eliminates the risk of false measured values by anti-bTSH-ab, which may be found in a significant extent in test samples.

This aspect of the present invention is also not made obvious from the prior art.

According to still another feature of embodiments of the present invention, an antibody binding human TSH is added as a reagent to the sample to be tested (*see*, *e.g.*, claim 29). Human TSH, if present in the sample to be tested, can adversely affect the assay since the human TSH will also bind to the hTSHR (in contrast, human TSH will not bind to solubilized porcine TSH receptor used in the conventional assay). Therefore, adding the antibody can help to avoid the disturbance of the assay by binding to any human TSH. This is also not described in the prior art.

In summary, prior to the discovery by the Appellant, as reflected in the claims on appeal, there did not exist, either in printed publications or in commercial embodiments, a solid-phase assay for determining with high specificity and high selectivity human TSH receptor autoantibodies. The cited prior art does not provide the directions for achieving this result.

Turning to the cited references, it is respectfully submitted that Vitti *et al.* (Acta Med Austriaca 23 (1-2): 52-56, 1996) (hereinafter "Vitti") actually teaches away from the assay of the present invention.

Briefly, Vitti concerns the interference of binding of TSH to the TSH receptor (TSHR) by autoantibodies to TSH receptor ("TRAb") in patients with autoimmune thyroid disease. There are two types of TRAb: those that mimic TSH and stimulate the thyroid, resulting in hyperthyroidism (thyroid-stimulating antibodies, or "TSAb") and those that inhibit the binding of TSH to TSHR (called TSH-blocking antibodies). Patients can also vary between hypothyroid and hyperthyroid, such as Graves' disease.

Vitti acknowledges that the TRAK assay had been used by "most laboratories" to measure TRAb. TRAK involves a competition assay, wherein autoantibodies bound to TSHR inhibit the binding of radiolabeled TSH to TSHR. TRAK is a radioreceptor assay, "RRA," since it uses a radioactive label bound to a receptor.

Vitti criticizes the TRAK assay (performed with porcine TSH receptor in a soluble preparation) because "it cannot distinguish between thyroid stimulating and TSH-blocking antibodies" since both inhibit binding of TSH but by different mechanisms.

Vitti took advantage of the recent cloning of the gene for human TSHR to develop a new *in vitro* bioassay for TSH autoantibodies that measure cAMP (cyclic adenosine monophosphate) production in Chinese hamster ovary (CHO) cells transfected with human TSHR (CHO-R cells). In this bioassay, stimulation of cAMP production measures hyperthyroidism (from TSAb) while inhibition of cAMP production indicates hypothyroidism (from TSH-blocking autoantibodies).

Thus, Vitti teaches a method of detecting TRAb's that is designed to distinguish between the two types (TSAb and TSH-blocking antibodies). Vitti asserts that this new method gave enhanced sensitivity over the TRAK assay.

However, those skilled in the art, understand that the bioassay of Vitti is very complicated and not very suitable for clinical use, *e.g.*, routine clinical diagnosis (*see* page 2, line 3 to page 3, line 8 of the specification). The bioassays disclosed by Vitti require living biological material, cannot be standardized (it being essentially impossible to compare numerical results obtained by different groups with different cells), are time consuming, require a separate detection of the cAMP released by the challenged cells into the culture medium, and are not suitable at all for commercial production of assays useful for clinical routine purposes. Due to the different measurement principle (cAMP concentration in culture medium as measure for the stimulation of the cells), the question as to whether the cells stimulated by the autoantibodies are present in liquid culture (as seems to be the case in Vitti) or attached to a surface, is, in fact, completely irrelevant to the measurement. This is, of course, in direct contrast to the presently claimed assay, in which the solid phase is used in the actual measurement.

Therefore, it is respectfully submitted that the practitioner of ordinary skill in the art would not have considered Vitti as a relevant source of information for developing or improving upon an assay, such as the prior commercially TBII assay, *e.g.*, TRAK assay, which is not a bioassay and which is viable for clinical procedures not merely in research laboratories or for specialized centers having their own in-house assays. Conversely, those

skilled in the art of the TBII assays would not turn to the bioassay methodology of Vitti for seeking improvements in, for example, the TRAK assay.

In either case, it should be specifically noted, that neither Vitti nor the TRAK assay use a solid phase. TRAK, is described in Vitti as being "solubilized," not solid ("the TSH receptor is contained in a solubilized membrane preparation," *see*, Vitti at page 55, second column, lines 1-2). The bioassay of Vitti is similarly carried out in tissue culture, *i.e.*, takes place in a Petri dish, but in no way is bound to the Petri dish. The Petri dish is not part of the assay and the TSHR is not bound to it.

Thus, the person of ordinary skill in the art would appreciate that Vitti teaches away from the embodiments of the present invention because Vitti advocates using a tissue-culture based bioassay to measure a molecule (cAMP) whose production is directly affected by both types of TRAb's, but in opposite ways. Vitti also abandoned the RRA assay of the TRAK method that was commonly used because it was unable to differentiate between types of TRAb.

Of course, it goes without saying that Vitti does not disclose a bioassay based on the competition of TSHR autoantibodies with TSH for binding sites of an immobilized functional TSHR preparation. Rather, as previously described, Vitti describes a bioassay based on stimulation of cAMP production in transformed cells; there is no competition with any labeled.

TSH (*e.g.*, ^{125}I-bTSH). Nor is there any dispute that Vitti does not disclose immobilizing rhTSHR to a solid phase, much less provide a discussion of how to avoid the sensitivity of TSHR's to undergo structural/conformational modifications as a result of immobilization, and especially, there is no disclosure or suggestion that certain monoclonal antibodies that recognize only conformational epitopes of hTSHR, in contrast to, for example, sequential monoclonal antibodies, can eliminate the sensitivity problem.

Neither Vitti's nor the TRAK bioassays are based on a solid-phase process in which the reacted solid phase is separated from the liquid biological sample since the reaction of all reagents is carried out in the liquid phase. At most, in the TRAK procedure, the separation of the solid phase (formed complex) from the liquid reaction mixture takes place after completion of the reaction of all reactants and this occurs by precipitating the formed

complex by addition of polyethylene glycol (PEG) (this is described on page 3, lines 9 *et seq.* of the present specification).

As such, it is respectfully submitted that the disclosure of Vitti is remote from the present invention and one of ordinary skill would not have been motivated by the disclosure of Vitti or any of the other cited prior art to make all of the modifications of the Vitti process necessary to arrive at the various claimed embodiments of the present invention, especially given the negative teachings with respect to solid phase RRA assays.

In the Advisory Action, it was suggested that Appellant's results "would be expected ... because of the use of recombinant human TSH receptor to detect human TSH autoantibodies instead of porcine TSH receptor to detect human autoantibodies in the conventional TRAK assay would improve the specificity of the assay as claimed" (*emphasis added*).

With all due respect, this assertion is without foundation or factual basis on the record. First, because the porcine receptor used in the TRAK assay is used in solubilized form, its replacement by a human receptor would still not result in a solid phase assay, but at best, merely another precipitation assay. Second, it is not clear why the replacement of porcine receptor with a human receptor would lead to a useful or better assay, for example, the additional possibilities of interfering sample components which do not disturb an assay using the porcine receptor. In any case, there is no evidence which supports the assertion, but even if correct, such substitution would still not at all suggest a solid phase assay nor would there be provided to the practitioner any knowledge of how to successfully immobilize the functional recombinant hTSH receptor (*i.e.*, without reducing the functionality by blocking binding sites, altering configuration, etc.).

The deficiencies of Vitti are not obviated by the remaining references.

Harlow, *et al.* (Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, pages 556, 564-591, 1988) (hereinafter "Harlow") is merely a background literature (text) reference which provides general details of the types of immunoassays, including, as in Table 14.1, page 558, protocols for choosing an assay protocol. There is no disclosure in Harlow of (1) a solid phase assay based on an affinity purified immobilized functional recombinant TSH receptor, as the binding agent in a competitive bioassay; (2) a two-stage process in which following the reaction of the solid phase with the liquid biological sample to be tested for the

TSH receptor autoantibodies, the reacted solid phase is separated and washed prior to incubating the reacted solid phase with a buffer solution comprising labeled competitor, namely, in this case, labeled bovine TSH; or (3) an immobilizing a receptor-type binding agent with a specialized linking agent that will not result in loss of functionality of the binding agent, much less, of course, of the use of monoclonal antibodies obtained by immunizing an animal with a DNA plasmid construct encoding the human TSH receptor and which recognize only conformational epitopes of the human TSH receptor.

Moreover, whereas the disclosure of Harlow appears to be used as evidence of the advantages of "antibody sandwich immunoassays," the process as set forth in the embodiments defined by the claims under appeal, are not sandwich immunoassays but, as described in detail, is a competitive receptor assay based on the competition of TSHR autoantibodies with labeled bTSH for a specific binder, which is a receptor.

Therefore, the combination of Vitti with Harlow does not support the rejection of claims 23-25 and 27-33 under 35 U.S.C. 103(a).

Nicholson *et al.* (J. Mol. Endocrinol. 16(2): 159-170, 1996) ("Nicholson") does not disclose or suggest a selective monoclonal antibody that recognizes only conformational epitopes of the human TSH receptor and certainly does not disclose or suggest that a monoclonal antibody that recognizes only conformational epitopes (and which is obtained by immunizing an animal with a DNA plasmid construct encoding the human TSH receptor) should be used for immobilizing a receptor to a solid surface in a bioassay for TSH receptor autoantibodies or that such monoclonal antibodies have any advantage relative to any other antibodies when used for such immobilization.

At most, Nicholson describes some monoclonal antibodies (m.Ab) to the extracellular domain of the human TSH receptor (TSH-R.E). The monoclonal antibodies were obtained by immunizing a mouse with a recombinant full or partial TSH-R.E obtained in insect cells (a baculovirus-insect cell expression system). As would be appreciated by the person of ordinary skill in the art, the TSH-R.E is not a functional TSH receptor, because they lack, *e.g.*, binding of TSH (*see, e.g.,* Nicholson at page 168, first column, lines 13-15).

There is no suggestion by Nicholson to use the mAbs to immobilize a functional human TSH receptor preparation or that doing so would provide an improved solid phase analogue of the known TRAK assay. Indeed, it is respectfully submitted that the evidence

does not support a conclusion that the mAbs disclosed by Nicholson recognize only conformational epitopes of human TSH receptor.

For example, in the case of the mAbs designated A10 and A11 (the numerical designations have no relationship, whatsoever, to the designations in Table 1 on page 28 of the subject application), Nicholson explains that these mAbs specifically bind to the same short peptide sequence comprising amino acids 22-35 of hTSH receptor. Therefore, these monoclonal antibodies are sequential antibodies.

Similarly, antibody A9 is said to recognize epitopes from the peptide sequence which includes aa 147-228; while antibody A7 is said to recognize epitopes from the peptide sequence which includes aa 402-415.

Of course, these antibodies were obtained by immunization with an antigen (non-functional human TSH-R.E derived from insect cells) in which the amino acids apparently are exposed and can, therefore, act as antigen. In contrast, in native hTSH-R, *e.g.*, in a thyroid section, the amino acid sequence comprising AA 22-35 seems to be hidden within the three-dimensional native receptor structure, such that antibodies A10 and A11 cannot bind to the sequence. By treating with acetone the native receptor is denatured, and the sequence then becomes accessible.

In any case, the mAbs described by Nicholson are not obtained by immunizing an animal with a DNA plasmid construct encoding the human TSH receptor. Rather, the mAbs of Nicholson are obtained by immunizing mice with a recombinant insect cell derived purified TSH-R.E peptide (*i.e.*, a partial TSHR peptide comprising only the amino acids of the extracellular domain of human TSHR). Such hTSH-R.E expressed in insect cells is <u>not functional</u>, as already recognized by Nicholson.

In contrast, immunization of an animal with a DNA plasmid construct will result in expression (weakly) of the injected foreign DNA, therefore, resulting in some native, apparently correctly-folded and, functional, human TSH-R, which can act as native antigen produced, *in vivo*, in the host animal.

As those skilled in the art will appreciate, there in no *a priori* presumption that immunizations with different antigens give the same antibodies. There is no *a priori* reason to assume that antibodies obtained by two different immunization methods will be the same. There is no *a priori* justification, therefore, for alleging that Nicholson discloses antibodies

that recognize only conformational epitopes of the human TSH receptor or that would be useful in solving the problem addressed in the present application, as reflected in the presently claimed embodiments.

Even if one of ordinary skill in the art would have a basis for assuming mAb A9 of Nicholson recognizes only conformational epitopes of the human TSH receptor (as well as a reason for making that assumption), it is respectfully submitted that the practitioner would still not have been motivated by the findings of Nicholson (*e.g.*, that, "[t]he availability of mAbs that recognize epitopes on different regions of the extracellular domain of TSH-R will lead to a better understanding of the autoantigenic regions on TSH-R implicated in disease activity" – *see*, Nicholson, at the Abstract), to use such mAb for the purpose of immobilizing a functional rhTSHR. But, if one did attempt to use mAb A9 for this purpose, he or she would realize that , as in the prior art attempts to immobilize TSH receptors, such mAb would not be able to achieve the immobilization without adversely affecting the functionality, for instance, the ability to selectively, and with high sensitivity, bind both all variants of human TSH receptor autoantibodies and the labeled bTSH.

Therefore, it is respectfully submitted that the combination of Vitti with Harlow and Nicholson do not provide evidence that the claimed subject matter would have been obvious to the person of ordinary skill in the art at the time the invention was made.

The disclosure of Morgenthaler *et al.* (J. Clin. Endocrinol Metab. 81(2): 700-706, Feb. 1996) (hereinafter "Morgenthaler") is essentially similar to the disclosure of Nicholson, insofar as apparently the same three mAbs, A7, A9 and A10, are described.

Moreover, in addition to failing to offer any suggestion that these mAbs provide any advantage (or could, in fact, be used) for the immobilization of functional hrTSHR and could do so without resulting in structural changes adversely impacting the functionality, Morgenthaler appears to use these mAbs in immunoprecipitation-type assays in which a preparation of an extracellular part of a recombinant TSH receptor labeled by incorporation of ^{35}S-methione is used as a reagent for the precipitation (see, "*Immunoprecipitation of in vitro translated TSH-R polypeptides with mAbs and polyclonal antisera,*" Morgenthaler at page 701, second column, to page 702, first column). In contrast, Morgenthaler used the TRAK assay for testing sera of patients for TBII activity (see, "*Patients and normal control sera,*" Morgenthaler at page 701, first column).

Accordingly, the combined disclosures of Vitti, Harlow and Morgenthaler do not provide evidence that the embodiments of the present invention under consideration and defined by claims 23-25 and 27-33, would have been obviousness at the time the invention was made.

Evidence of Non-obviousness

Furthermore, none of the cited references provide any evidence of successfully obtaining the presently claimed invention, let alone its superior results. As evidence of the superior and unexpected nature of the presently claimed invention, reference is made to Costagliola *et al.*,[1] which provides a comparison of the conventional radioreceptor assays (*e.g.*, the TRAK assay) and those of the present invention (*e.g.*, the TRAKhuman assay). As can be seen from the abstract "a specificity of 99.6% with a sensitivity of 98.8%" was obtained for the solid state assays, while a "99.6% specificity and 80.2% sensitivity" was obtained for the solution phase assay. The enhanced specificity (98.8% versus 80.2%) is not suggested in and could not have been expected from the cited art.

Claims 23, 25/23, 28, 29/23, 30/23, 31/23, 32/23, and 33, are separately patentable over the prior art

Furthermore, with respect to the embodiment of the invention as set forth in claim 23 and the claims dependent thereon, Vitti does not disclose a two-stage bioassay, and such deficiency is not obviated by the secondary/tertiary references.

Therefore, claims 23, 25/23, 27/23, 28, 29/23, 30/23, 31/23, 32/23, and 33, are separately patentable over the prior art, and reversal of the rejection of these claims is respectfully requested.

Rejection of claims 23-25 and 27-33 under 35 U.S.C. 103(a) as unpatentable over U.S. 5,614,363 in view of Vitti and Harlow in view of Nicholson or Morgenthaler

As stated at the outset, it is assumed that the rejection over the disclosure of U.S. 5,614,363 (Cook *et al.*), in view of Vitti, Harlow, and Nicholson or Morgenthaler, which

[1] J. Clin. Endocrinol. Met., 84(1), 90-97 (1999). A copy of this literature is of record.

rejection was not discussed in the Advisory Action Before the Filing of an Appeal Brief, has been withdrawn from the final rejection.

If not, however, such rejection is respectfully submitted to be in error and should be withdrawn.

In this regard, US 5,614,363, ("'363 patent") is cited for its disclosure at Col. 7, lines 8-63 and Col. 8, lines 3-11, outlining competitive binding assays employing radiolabeled TSH and TSH receptor.

Regarding the discussion in Col.7, the '363 patent proposes a purification strategy for the TSH receptor protein comprising several solubilization and affinity chromatography steps. A TSH receptor protein which undergoes such purification steps does not retain its functionality (if the purified recombinant TSH receptor was a functional TSH receptor at all – which depends, *inter alia,* from the cells used for its expression).

Regarding the passage at Col. 8, this discussion in the '363 patent, **at best**, suggests only a use of the assay, but hardly enables a skilled artisan to develop a useful solid phase assay for the detection of the claimed antibodies in biological liquid samples of patients. It is noted that conventional assays, at the time of the '363 patent, used solubilized porcine TSH receptors almost exclusively. Therefore, this discussion could not fairly be cited as evidence of a suggestion of the solid phase techniques of the present invention.

Therefore, even if the disclosure of the '363 patent is considered in combination with the disclosures of Vitti, Harlow, and Nicholson or Morgenthaler, these combined disclosures do not provide evidence that the subject matters as set forth in the rejected and appealed claims 23-25 and 27-33, would have been obvious to a person of ordinary skill in the art at the time the invention was made.

Therefore, for any and all of the reasons set forth herein, the rejection(s) of claims 23-25 and 27-33, under 35 U.S.C. § 103(a), should not be sustained, and should be reversed by the Honorable Board of Patent Appeals and Interferences.

Respectfully submitted,

MAYER BROWN ROWE & MAW LLP

By: 

Richard A. Steinberg
Registration No. 26,588
Direct No. (202) 263-3325

Paul L. Sharer
Registration No. 36,004
Direct No. (202) 263-3340

PLS/RAS
Intellectual Property Group
1909 K Street, N.W.
Washington, D.C. 20006-1101
(202) 263-3000 Telephone
(202) 263-3300 Facsimile

Date: July 25, 2005

APPENDIX OF CLAIMS

1-12. (Cancelled).

13. (Withdrawn).

14-22. (Cancelled).

23. A method for the determination of TSH receptor autoantibodies comprising:

(i) reacting a solid phase, comprising an affinity-purified immobilized functional recombinant human TSH receptor, with a liquid biological sample to be assayed for the presence of said autoantibodies;

(ii) separating a reacted solid phase from the liquid biological sample;

(iii) washing the reacted solid phase;

(iv) incubating the reacted solid phase with a buffer solution comprising an amount of labeled bovine TSH for a sufficient time to occupy all the TSH binding sites of the functional recombinant human TSH receptor not occupied by the autoantibodies; and

(v) determining the presence and/or amount of the autoantibodies on the basis of the amount of labeled bovine TSH bound to the solid phase;

wherein the functional recombinant human TSH receptor is immobilized to a solid support by a selective monoclonal antibody that recognizes only conformational epitopes of the human TSH receptor and is obtained by immunizing an animal with a DNA plasmid construct encoding the human TSH receptor and washed.

24. A method for the determination of TSH receptor autoantibodies comprising:

(i) reacting a solid phase comprising an affinity-purified immobilized functional recombinant human TSH receptor with a solution prepared from:

a) a serum-containing biological sample to be assayed for the presence of said autoantibodies, and

b) a buffer solution containing an amount of labeled bovine TSH for a sufficient time to occupy all the TSH binding sites of the functional recombinant human TSH receptor not occupied by the autoantibodies;

(ii) separating the solution from a reacted solid phase;

(iii) washing the reacted solid phase; and

(iv) determining the presence and/or amount of the autoantibodies on the basis of the amount of labeled bovine TSH bound to the solid phase;

wherein the functional recombinant human TSH receptor is immobilized to the solid support by a selective monoclonal antibody that recognizes only conformational epitopes of the human TSH receptor and is obtained by immunizing an animal with a DNA plasmid construct encoding the human TSH receptor and washed.

25. The method according to either claim 23 or claim 24, wherein the solid phase is formed by the walls of test tubes, which are pre-coated with an animal-specific antibody for binding the selective monoclonal antibody against the human TSH receptor.

26. (Cancelled).

27. The method according to either claim 23 or claim 24, carried out in an automated form, wherein the solid phase comprises suspended particles that are coated with ~~a~~ said selective monoclonal antibody against the human TSH receptor, and wherein by adding the functional recombinant human TSH receptor and the sample a solution containing the suspended solid particles and the receptor is temporarily formed.

28. The method according to claim 23, wherein the labeled bovine TSH is added in a serum-free buffer solution.

29. The method according to either claim 23 or claim 24, wherein step (i) is carried out in the presence of at least one antibody against human TSH that does not cross-react with bovine TSH.

30. The method according to either claim 23 or claim 24, wherein the autoantibodies are receptor-stimulating autoantibodies, whose occurrence in a human serum is characteristic of Graves' disease.

31. The method according to either claim 23 or claim 24, wherein the affinity-purified immobilized functional recombinant human TSH receptor is in the presence of a buffer.

32. The method according to either claim 23 or claim 24, wherein said sample is diluted with a buffer.

33. A method for the determination of TSH receptor autoantibodies comprising:

(i) reacting a solid phase, comprising an affinity-purified immobilized functional recombinant human TSH receptor, with a liquid biological sample to be assayed for the presence of said autoantibodies;

(ii) separating a reacted solid phase from the liquid biological sample;

(iii) washing the reacted solid phase;

(iv) incubating the reacted solid phase with a buffer solution comprising an amount of labeled bovine TSH for a sufficient time to occupy all the TSH binding sites of the functional recombinant human TSH receptor not occupied by the autoantibodies; and

(v) determining the presence and/or amount of the autoantibodies on the basis of the amount of labeled bovine TSH bound to the solid phase;

wherein the functional recombinant human TSH receptor is immobilized to a solid support by a selective monoclonal antibody that:

a) recognizes only conformational epitopes of the functional human TSH receptor and is obtained by immunizing an animal with a DNA plasmid construct encoding the human TSH receptor; and

b) does not bind to peptides representing short sequences of the human TSH receptor, but which shows strong binding to the complete functional recombinant human TSH receptor;

and then washed.

EVIDENCE APPENDIX

1. Costagliola *et al.*, J. Clin. Endocrinol. Met., 84(1) 90-97 (1999).

RELATED PROCEEDINGS APPENDIX

None.

APPENDIX FOR RULES & STATUTES

None.